A COMPARISON OF THE RESISTANCE OF WHITE LEGHORN AND WHITE MINORCA CHICKENS TO ASCARIDIA LINEATA (SCHNEIDER)

by

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INTRODUCTION

The experimental studies of Dr. J. E. Ackert and his associates on the varied phases of resistance of chickens to the large roundworm, Ascaridia lineata (Schneider) were initiated in 1921 by Ackert and Herrick (1928) who demonstrated a definite resistance of chickens to this parasite. A great deal of the subsequent work has dealt with resistance factors. Herrick (1926) demonstrated that a gradually increasing age resistance to A. lineata was developed in chickens raised in confinement. Ackert (et al) (1929, 1931a, 1931b, 1933) have shown the relationship of certain vitamins and dietary supplements to the resistance of chickens to this nematode. The work of Graham, Ackert, and Jones (1932) indicates the development of an acquired resistanse of chickens to this parasite. Porter and Ackert (1933) demonstrated that the resistance of chickens to the intestinal nematode, A. lineata may be lowered by the repeated loss of blood.

Investigators in other fields have studied resistance and its development. Probably a great deal more work has been done with bacteria than with the protozoa and the metazoa. The investigations of Roberts and Card (1926) demonstrated a natural resistance to bacillary White Diarrhea that was hereditary. Lambert (1932) working with a bacterial disease of chickens, supplied evidence of inherited resistance. Blacklock and Gordon (1927) demonstrated a true metazoan immunity in their work with parasitic dipterous larvae. Among the helminthologists, Herrick (1928) showed evidence of the development of age resistance in his studies of the dog hookworm, Ancylostoma caninum, but he was unable to demonstrate an immunity traceable to previous infection. McCoy (1931) working with the same parasite believed that such an acquired immunity was developed. Sandground (1928) demonstrated the development of a specific immunity in the case of a nematode infection of dogs and cats. Rebrassier and McCrory (1931) failed to show significant evidence of an artificial immunity to Ascaridia lineata in chickens.

Relatively little work has been done on the comparable resistance of races or varieties to helminths. It is well known that the various races of the human family are not equally susceptible to certain bacterial and protozoan diseases. Roberts and Card (1926) compared the resistance of White Plymouth Rock and Rhode Island Red chickens to bacillary white diarrhea. While they did not find a significant difference between the breeds, their work indicates a varied resistance in strains in the respective breeds. Lambert's work (1932) suggests differences in resistance among strains. Harris and Boughton (1928) and Dudley (1928) have studied the death rates of various standard breeds of fowl. No essentially different death rate was shown in these comparisons.

In studying factors in the resistance of chickens to the nematode, A. lineata, during the last few years, Dr. J. E. Ackert and his associates have used pure bred White Leghorn chickens, almost exclusively, as the host animals. To ascertain if other breeds react similarly to the

A. lineata, a series of five experiments were begun in October, 1932, and terminated in March, 1934, comparing the resistance of White Leghorn and White Minorca chickens to the fowl nematode, Ascaridia lineata (Schneider).

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MATERIALS AND METHODS

The general plan of this problem was to subject White Leghorns and White Minorcas to equivalent experiments to determine the comparative resistance of these two Mediterranean breeds of Gallus domesticus to the large fowl nematode, Ascaridia lineata (Schneider). Pure bred chickens obtained from an accredited hatchery were used in all of the experiments. Both breeds were from the same hatches and were about thirty-six hours old when received. A probable variance in age resistance and other factors was thus controlled.

The chickens were raised helminth-free, in clean, well-lighted, well-ventilated, rat-proof pens. The breeds were provided with equivalent floor space in cement-floored pens covered with clean straw. Sanitary conditions were maintained. An adequate diet for chickens reared in confinement, was fed in hoppers and was before the chickens at all times. It consisted of the following ingredients: yellow corn meal, 40 parts; alfalfa leaf meal, 4 parts; meat meal, 10.4 parts; skim milk powder, 6.4 parts; oatona, 12 parts; cracked wheat, 15 parts; and cod liver oil, 1.64 parts.

At approximately two weeks of age, the chickens were banded and weighed individually. Thereafter they were weighed each week until the termination of the respective experiments. The average weights were calculated for each breed of chickens and growth curves were plotted to determine the comparative rates of growth. In Experiments 1, 2 and 3, groups of the two breeds were parasitized at ages of approximately one month and six weeks, respectively. In Experiments 4 and 5 the breeds were parasitized at 12, 19, 26, 33, and 40 days, respectively.

The life cycle of *A. lineata* is direct; the embryonated egg is the infective stage (Ackert, 1931). Gravid female *A. lineata* were obtained from a large poultry packing house. The viscera of the worms of approximately the same size were removed and the uteri isolated and punctured to secure samples of the eggs. Ackert (1931) showed that more fertilized eggs were found in the proximal portions of the uteri and that the presence of a central light spot was indicative of the fertility of the egg. These eggs were placed in sterile petri dishes, in distilled water with four to five drops of 2 per cent formalin as a protective measure against molds and bacteria. Extraneous material such as pieces of uteri and other tissues were removed from the petri dishes. The cultures were carefully stirred at 48 to 72 hour intervals to allow sufficient oxygen to reach the eggs. The latter were incubated at temperatures between 27° and 30° C. for approximately three weeks. A steady temperature approaching the optimum (30 to 33° C.) is necessary for an even development of the eggs (Ackert, 1931). Care was taken to have the maximum number of eggs in the infective stage upon the day of parasitizing.

It was determined to use 50 ± 5 embryonated eggs of *A. lineata* as the dosage. Certain advantages accrue in the use of this number. It has been shown that 50 eggs provide a reasonable and satisfactory per cent of developing worms (Ackert, Graham, Nolf, and Porter, 1931). The number is small enough to be conveniently counted -- an important time factor in the experimental infection of a large number of hosts. Another probable advantage is that this number of worms will not cause a heavy mortality among the chickens.

The counting of 50 ± 5 embryonated eggs was accomplished with the aid of a compound microscope and a mechanical stage. A drop of egg culture was placed upon a glass slide and the number of embryonated eggs counted. Other drops of culture were treated likewise until the total number of infective eggs lay between the 45 to 55 limits. Then these drops were carefully wiped up with small pieces of filter paper and these were placed in the esophagus of the chicken with the aid of blunt forceps.

The period of parasitism was terminated in three weeks, because by that time the worms have withdrawn from the submucosa and are living free in the intestinal lumen (Ackert, 1923). Shortly after this age, they begin to be eliminated from the intestine in increasing numbers (Ackert and Herrick, 1928). To minimize the intestinal contents and to facilitate collections of the worms all feed and litter were removed from the pens several hours before the birds were killed. The chickens were then sacrificed and the intestines from the gizzard to the c caeca removed. The worms and debris were flushed from the intestines with the aid of hot water under pressure (Ackert and Wolf, 1929). The contents of the intestines thus secured, were placed in Mason jars in 5 per cent formalin with the leg band of the chicken for identification. A binocular microscope which could be rotated on a movable arm, was used in the search for worms. When _A. lineata_ were found, they were placed in small vials in 2 per cent formalin with the respective leg band.

As an aid in measuring the worms, use was made of a photographic enlargement apparatus. Shadows of the worms enlarged exactly six times were projected and traced upon onion skin paper. A milled wheel calibrated for a direct reading was run the length of the traced line. The criteria for judging the resistance were the number (viability) and the length (growth) of the _A. lineata_ from each group of chickens under comparison. The ratio D/E (actual difference divided by the error of the difference) was determined statistically for both numbers and lengths of worms to reveal possible significance in the comparisons of the breeds of White Leghorns and White Minorcas.

In this paper a ratio of 3.00 or more is considered indicative of a significant difference.

EXPERIMENTAL DATA

Experiment 1

Fifty White Leghorn and fifty White Minorca chickens hatched at an accredited commercial hatchery October 2, 1932, were received within 36 hours. At 16 days of age these chickens were banded and weighed. Weekly average weights were determined throughout each experiment and the growth curves of the two breeds compared (Fig. 1). This chart shows that while the White Leghorns averaged slightly heavier during the first few weeks, the White Minorcas forged ahead at approximately four weeks of age and from then on they slowly built up an ever-increasing lead.

The growth charts of Experiments 2 and 3 illustrate similar curves. In Experiment 2, the growth curves correspond very closely to those of Experiment 1 (Fig. 2). In Experiment 3, the growth curves for both breeds are rather erratic (Fig. 3). The Minorcas are the heavier breed throughout. While this chart does not correlate closely with Figures 1 and 2 (which can very nearly be superimposed upon each other), yet the general trend is very similar.

Group C. On November 1, 1932, at the age of 30 days,

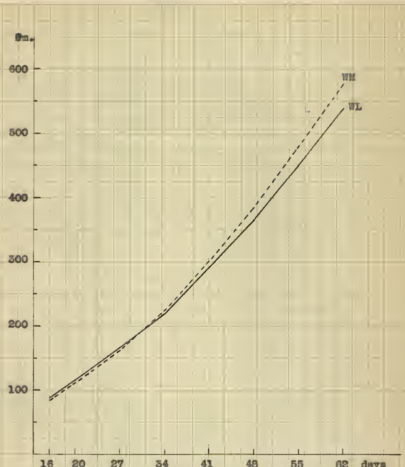

Fig. 1. Showing growth curves of White Leghorns (WL) and White Minorcas (WM). Fall, 1932.

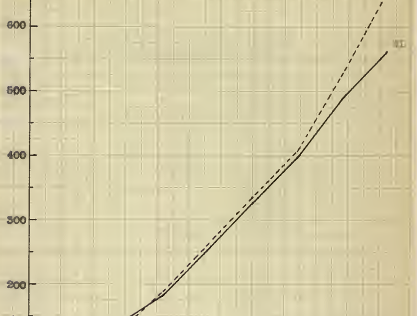

Fig. 2. Showing growth curves of White Leghorns (WL) and White Minorcas (WM). Winter, 1933.

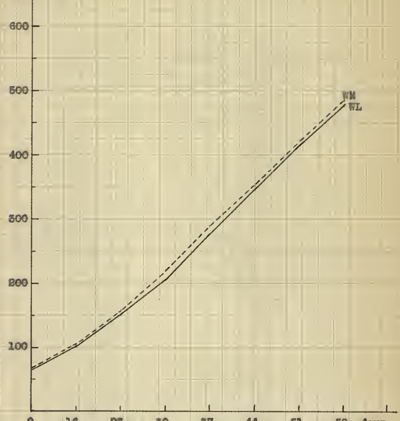

Fig. 3. Showing growth curves of White Leghorns (WL) and White Minorcas (WM). Summer, 1933.

24 White Leghorn and 24 White Minorca chickens chosen at random from the original groups were placed arbitrarily in Group C and were parasitized with 50 ± 5 embryonated eggs of A. lineata. After a period of parasitism of three weeks duration, these chickens (age 51 days) were killed. The intestines were immediately removed and flushed and their contents placed in Mason jars. Upon isolating the A. lineata it was discovered that the range of infestation was one to 22 worms in the White Leghorns with five chickens escaping infestation; and one to seven worms in the Minorcas with ten chickens free from A. lineata. The average number of worms for the Leghorns was 5.33; for the Minorcas, 1.76, or a significant difference of 3.57 worms per chick (4.38 times the probable error), Table 1. In regard to the lengths of the worms, those in the Leghorns averaged 22.29 mm., while those in the Minorcas averaged 16.5 mm., a mean difference of 5.79 mm., which was significant (7.34 times the probable error). According to this analysis, the White Minorcas were decidedly more resistant to infestation with the nematode, A. lineata, than were the White Leghorns.

Group E. On November 15, 1932, at the age of 44 days, the remaining chickens of Experiment 1 (24 Leghorns and 23 Minorcas) were placed in Group E and parasitized with 50 ± 5 embryonated eggs of A. lineata. As before, the worms were allowed three weeks for growth. Then the chickens

Table 1. Comparison of Ascaridia lineata found in chickens of Experiment 1, hatched October 2, 1932; Group C parasitized at four weeks of age; Group E parasitized at six weeks of age, and killed after three weeks parasitism, respectively.

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio D / P.E.D.
Numbers of Worms	W. Leghorns	C	24	5.33	5.55	.766	3.57	.816	4.38
	W. Minorcas	C	25	1.76	2.10	.284			
	W. Leghorns	E	24	1.67	1.59	.219	0.23	.327	0.70
	W. Minorcas	E	23	1.43	1.72	.242			
Lengths of Worms (mm.)	W. Leghorns	C	24	22.29	6.83	.408	5.79	.789	7.34
	W. Minorcas	C	25	16.5	6.63	.529			
	W. Leghorns	E	24	17.31	5.59	.597	1.07	.797	1.34
	W. Minorcas	E	23	18.38	4.5	.529			

(age 65 days) were killed and the worms obtained subjected to biometrical analysis. The range of infestation for the Leghorns was one to seven worms, with six chickens escaping infestation; and for the Minorcas, one to six worms with ten chickens free from A. lineata. The average number of worms per Leghorn was 1.67, per Minorca, 1.43; with a mean difference of 0.23 worms which was not significant. In considering the lengths of worms, those in the White Leghorns averaged 17.31 mm. and those in the Minorcas slightly longer (18.38 mm.), a mean difference of 1.07 mm., which was not significant.

Herrick (1926) showed that experimental chickens (White Leghorns) developed age resistance. Certain phases of these experiments confirm his work (Table 2). The Group C Leghorns averaged 5.33 worms per chick, while Group E averaged 1.67 nematodes which gives a significant difference of 3.66 worms per chick (4.60 times the probable error). The average length of the worms in the younger chickens (Group C) was 22.29 mm., as compared with 17.31 mm. in Group E Leghorns. The mean difference of 4.98 mm. is 6.89 times the probable error and therefore considered significant. It is evident from these data, that the White Leghorns have developed considerable age resistance in the two weeks intervening between Group C and Group E.

Table 2. A summary of the numbers and lengths of the Ascaridia lineata from the chickens of Experiment 1 (Fall, 1932) for evidences of age resistance.

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio D / P.E.D.
Numbers of Worms	W. Leghorns	C	24	5.33	5.55	.766	3.66	.796	4.60
	W. Leghorns	E	24	1.67	1.59	.219			
	W. Minorcas	C	25	1.76	2.10	.284	0.33	.373	0.88
	WhiMinorcas	E	23	1.43	1.72	.242			
Lengths of Worms (mm.)	W. Leghorns	C	24	22.29	6.83	.408	4.98	.723	6.89
	W. Leghorns	E	24	17.31	5.59	.597			
	W. Minorcas	C	25	16.5	6.63	.675	1.88	.857	2.19
	W. Minorcas	E	23	18.38	4.5	.529			

In considering the White Minorcas, Group C had an average of 1.76 worms per chick; Group E an average of 1.43 worms, a mean difference of 0.33 which was not significant. The average length of the worms in the Group C Minorcas was 16.5 mm. compared with a slight increase in average length for Group E (18.38 mm.). The average difference of 1.88 mm. was not significant.

The results of this experiment indicate that of the chickens parasitized at 30 days, the White Minorcas are more resistant to infestation than are the White Leghorns; that the Leghorns develop a decided age resistance in the interval between the respective groups, and that the Minorcas apparently do not develop an increased resistance to infestation.

Experiment 2

Fifty White Leghorns and fifty White Minorcas hatched February 6 were received February 8, 1933. The same technique was employed with these chickens as with those of Experiment 1. Weekly average weights were charted, the growth curves for the chickens of Experiment 2 (Fig. 2) closely paralleling those of Experiment 1.

Group C. At 29 days of age (March 7, 1933) twenty-five chickens of each breed were parasitized with 50 ± 5 embryonated eggs of *A. lineata.* The viability of these eggs was accidentally destroyed shortly before these chickens were parasitized, so that when the chickens were killed three weeks later, no worms were found.

Group E. The remaining chickens of Experiment 2, constituting 22 White Leghorns and 20 White Minorcas were parasitized at the age of 43 days, two weeks after those of Group C. Three weeks later, these chickens (age 64 days) were killed and the worms counted and measured. These data were subjected to biometrical analysis as in the previous work (Table 3). The range of infestation for the Leghorns was one to six worms; for the Minorcas, one to five worms. Five Leghorns and twelve Minorcas escaped infestation. The average number of worms per Leghorn chick was 2.36; per Minorca, 0.9, a mean difference of 1.46 which was not significant. The average length of the worms in the Leghorns was 24.63 mm. as compared with 16.5 mm. for the Minorcas. The average difference of 8.13 is significant (7.90 times the probable error). This analysis indicates that the Minorcas are more resistant to the growth of the *A. lineata* than are the White Leghorns.

Table 3. Comparison of *Ascaridia lineata* found in chickens of Experiment 2, hatched February 6, 1933; parasitised March 21, 1933, and killed at nine weeks of age after three weeks of parasitism.

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio $\frac{D}{P.E.D.}$
Numbers of Worms	W. Leghorns	E	22	2.36	1.85	.266	1.46	1.077	1.36
	W. Minoreas	E	20	0.9	1.41	.213			
Lengths of Worms (mm.)	W. Leghorns	E	22	24.63	6.402	.599	8.13	1.029	7.90
	W. Minoreas	E	20	16.5	5.26	.837			

Experiment 3

White Leghorn and White Minorca chickens hatched May 7, 1933, were received the following day. Average weekly weights of the respective breeds were graphed (Fig. 3). While the Minorcas were slightly heavier from the start and the respective growth curves were somewhat erratic, comparison with Figures 1 and 2 of the previous experiments, respectively, will show a related trend.

Group C. At the age of 30 days (June 6, 1933) 25 Leghorns and 24 Minorcas were parasitized with 50 ± 5 embryonated eggs of A. lineata. Conforming with previous work, the nematodes were allowed three weeks for growth, at which time the hosts (age, 51 days) were killed and the worms secured for biometrical analysis (Table 4). The range of infestation for the Leghorns was one to 37 worms with no infestation found in five chickens. For the Minorcas, the range was one to 14 worms with 12 chickens escaping infestation. The average number of worms for the Leghorns was 6.12 compared with a 3.3 average for the Minorcas, a mean difference of 2.82, which is 2.45 times the probable error, just short of significance. Taken alone it would mean nothing, but in confirmation of breed comparisons of Experiment 1, it suggests that the difference

Table 4. Comparison of Ascaridia lineata found in chickens of Experiment 3, hatched May 7, 1933; parasitized June 6, 1933, and killed at seven weeks of age after three weeks of parasitism.

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio D / P.E.D.
Numbers of Worms	W. Leghorns	C	25	6.12	7.94	1.071	2.82	1.152	2.24
	W. Minorcas	C	24	3.3	3.07	.424			
Lengths of Worms (mm.)	W. Leghorns	C	25	26.07	5.79	.316	5.63	.534	10.55
	W. Minorcas	C	24	20.44	5.66	.430			

between the respective breeds is valid and not due simply to chance. In regard to length of worms, the average for the Leghorns was 26.07 mm., that for the Minorcas 20.44 mm., a mean difference of 5.63 which is 10.55 times the probable error of the difference. These results confirm those of Experiment 1.

Group E. According to the schedule, chickens of Group E were to be parasitized June 20, 1933, at 44 days of age. Mold had destroyed suitable egg cultures of A. lineata so that this group could not be parasitized to obtain further statistical evidence.

Combined Results of Experiments 1, 2 and 3

The results of Experiments 1, 2 and 3 dealing with two groups of chickens were combined and the data treated statistically (Table 5). There were two acceptable C groups (Experiments 1 and 3). Likewise there were two acceptable E groups (Experiments 1 and 2).

Group C (Combined). The 49 White Leghorns averaged 5.73 worms per chick, and 49 White Minorcas 2.51 nematodes, a mean difference of 3.22 which was significant (4.52 times the probable error). The worms from the Leghorns averaged 24.35 mm. in length while those from the Minorcas averaged 19.03 mm., a significant difference of 5.32 which was 11.41

Table 5. A summary of the Ascaridia lineata found in chickens of Experiments 1, 2 and 3 (Fall, 1932; Winter, 1933; and Summer, 1933, respectively).

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio D / P.E.D.
Numbers of Worms	W. Leghorns	C	49	5.73	6.88	.663	3.22	.713	4.52
	W. Minorcas	C	49	2.51	2.73	.263			
	W. Leghorns	E	46	2.0	1.76	.175	0.81	.240	3.37
	W. Minorcas	E	43	1.19	1.60	.165			
Lengths of Worms (mm.)	W. Leghorns	C	49	24.35	6.56	.264	5.32	.466	11.41
	W. Minorcas	C	49	19.03	6.32	.384			
	W. Leghorns	E	46	21.45	7.07	.497	3.74	.678	5.52
	W. Minorcas	E	43	17.71	4.88	.461			

times the probable error.

Group E (Combined). The 46 Leghorn hosts averaged 2.0 worms per chick, and 43 Minorcas 1.19 *A. lineata*, an average difference of 0.81 which was 3.37 times the probable error. In the Leghorns, the average length of the worms was 21.45 mm. as compared with 17.71 mm. in the Minoreas, a mean difference of 3.74 which was significant (5.52 times the probable error).

Age Resistance (Combined). The Group C Leghorns averaged 5.73 worms per chick, while the older birds (Group E) averaged 2.0 worms per host. The mean difference in numbers between the two groups was 3.73, which is 5.44 times the probable error and therefore considered significant. The average length of the worms in Group C was 24.35 mm. and in the older Leghorn chickens 21.45 mm., a mean difference of 2.90 which was significant (5.15 times the probable error). Among the Group C Minorcas the average number of worms was 2.51; in the Group E Minorcas the average was 1.19 nematodes per chick, a mean difference of 1.32 which was significant (4.25 times the probable error). The average length of the worms in the C group was 19.03 mm. and in the older group of this breed it was 17.71 mm., an average difference of 1.32 mm. which was not significant (2.2 times the probable error).

The difference between the two breeds in resistance to infestation with the roundworm, A. lineata, was very marked. The White Minoreas were consistently more resistant to the A. lineata than were the White Leghorns. The ratios between the breeds in Group C are greater than are the respective ratios in Group E. The combined results show that both breeds have developed age resistance. This is far more marked for the Leghorns than in the Minorcas (Table 6). The Leghorns show significant differences for both numbers and lengths of A. lineata. The Minorcas show a significant difference in numbers between the two groups, but the difference in lengths is not constant.

Experiment 4

The White Leghorn and White Minorca chickens used in Experiment 4 were hatched at an accredited commercial hatchery October 15, 1933, and were received October 17, 1933. These chickens were reared under conditions similar to those of the previous experiments and the same technique was employed throughout. Average weekly weights were taken and the growth curves of the two breeds plotted (Fig. 4). In comparing this growth chart with those of previous experiments (Figs. 1, 2 and 3) a marked discrepancy is at once noticed. The White Minoreas, according to the standard

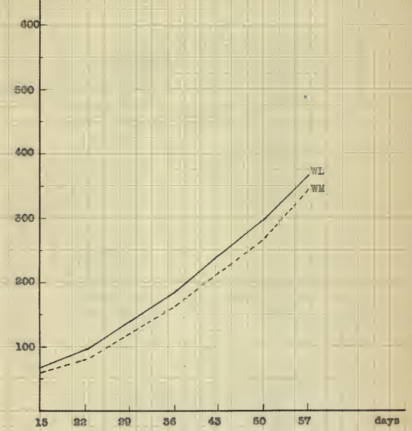

Fig. 4. Showing growth curves of White Leghorns (WL) and White Minorcas (WM). Fall, 1933.

Table 6. A summary of the numbers and lengths of the *Ascaridia lineata* from the chickens of Experiments 1, 2, and 3 (Fall, 1932; Winter, 1933; and Summer, 1933, respectively) for evidences of age resistance.

Breed	Group	Number of hosts	Average number worms	Dif.	Ratio	Average length worms	Dif.	Ratio
W. Leghorns	C	49	5.73	3.73	5.44	24.35	2.90	5.15
	E	46	2.0			21.45		
W. Minorcas	C	49	2.51	1.32	4.25	19.03	1.32	2.20
	E	45	1.19			17.71		

weights of breeds (Jull, 1926) are listed as one pound heavier than the Leghorns and it is reasonable to assume that, starting at approximately the same weights, the Minorcas would build up a slight but ever-increasing lead on the basis of normal growth curves. The respective growth charts (Figs. 1, 2 and 3) of Experiments 1, 2 and 3 exemplify the normal growth curves, but with Experiment 4 this is not the case. In this experiment the growth curve of the Minorcas is well below that of the Leghorns, a discrepancy which seems to be closely correlated with the results obtained in the biometrical analysis of the data on the parasites in this experiment (Table 7). These results indicate that different strains of Minorcas if not of Leghorns were used in Experiment 4.

Group A. At 12 days of age 23 Leghorns and 21 Minorcas (October 27, 1933) were parasitized with 50 ± 5 embryonated eggs of A. lineata. After a period of parasitism of three weeks duration, these chickens were killed at the age of 33 days. In isolating the A. lineata it was found that of the 44 chickens only one, a Minorca, completely escaped infestation. The range of infestation was one to 12 worms in the Leghorns, and one to 16 A. lineata in the Minorcas. The average number of nematodes per Leghorn was 5.5; per Minorca, 8.1, or a difference of 2.6 worms per chick which

Table 7. Comparison of *Ascaridia lineata* found in chickens of Experiment 4, hatched October 15, 1933; Group A parasitized at 12 days, Group B at 10 days, Group C at 26, and Group E at 40 days of age; and killed after three weeks of parasitism, respectively.

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio D/P.E.D.
Numbers of Worms	W. Leghorns	A	23	5.5	3.68	.518	2.6	.784	3.32
	W. Minorcas	A	21	8.1	4.0	.589			
	W. Leghorns	B	24	10.9	7.57	1.042	.2	1.415	1.41
	W. Minorcas	B	22	10.7	6.66	.958			
	W. Leghorns	C	25	11.0	9.53	1.27	1.2	1.88	.638
	W. Minorcas	C	24	9.8	10.09	1.39			
	W. Leghorns	E	18	4.5	4.23	.673	2.55	.748	3.41
	W. Minorcas	E	20	1.95	2.16	.326			
Lengths of Worms (mm.)	W. Leghorns	A	23	19.63	6.89	.414	.85	.576	1.52
	W. Minorcas	A	21	18.78	7.20	.374			
	W. Leghorns	B	24	20.01	5.87	.245	.58	.361	1.61
	W. Minorcas	B	22	20.59	6.02	.265			
	W. Leghorns	C	25	17.5	6.04	.246	1.3	.388	3.35
	W. Minorcas	C	24	18.8	6.84	.300			
	W. Leghorns	E	18	13.09	5.19	.389	2.26	.603	3.75
	W. Minorcas	E	20	10.83	4.27	.461			

probably was significant (3.32 times the probable error). According to this analysis, the Minorcas were less resistant to infestation than were the Leghorns. In considering the lengths of the worms, those in the Leghorns averaged 19.63 mm., while those in the Minorcas averaged 18.78 mm. per nematode, a mean difference of 0.85 mm. which was not significant. It will be seen that the percentage of infestation was very high as evidenced by only one chicken out of 44 with no worms and that variation in numbers of worms is not marked, two factors suggesting the existence of comparatively well-marked strains in the respective breeds.

Group B. On November 3, 1933, at 19 days of age, 24 Leghorns and 22 Minorcas were parasitized with 50 ± 5 embryonated eggs of the nematode, A. lineata. The parasites were allowed three weeks for growth, and then the hosts were sacrificed and their intestinal contents examined for worms. The range of infestation was from one to 25 worms in the White Leghorns with two chickens free from infestation. In the Minorcas, the numbers of worms ranged from one to 23, only one chicken escaping infestation. The average number of nematodes in the Leghorns was 10.9 while in the Minorcas, the average was 10.7. The difference of 0.2 was negligible. As to the length of the parasites the Leghorns averaged 20.01 mm. as compared with 20.59 mm. for the Minorcas. The mean difference of 0.58 was not significant. Summarizing, the percentage of infestation was quite high for both breeds as it was in Group A of this experiment. On the basis of the statistical analysis of the data from Group B the two breeds seem to be about equally susceptible to infestation with this parasite.

Group C. The 25 Leghorns and 24 Minorcas of Group C were parasitized November 10, 1933, at 26 days of age with 50 ± 5 embryonated eggs of *A. lineata.* Three weeks later the chickens (age 57 days) were killed and the worms isolated as in previous experiments. Each breed showed a range of infestation of one to 39 worms and also in each breed four chickens escaped infestation. The average number of worms per White Leghorn was 11.0, per Minorca 9.8, a mean difference of 1.2 worms which was not significant. The average length of the Leghorn parsaites was 17.5 mm. while those in the Minorcas were slightly longer, averaging 18.8 mm., a mean difference between the breeds of 1.3 mm. which was significant (3.35 times the probable error). This analysis suggests that the Minorcas are less resistant to infestation than are the Leghorns. Again, it is to be noted that the percentage of infestation was quite high and that variation in numbers of worms between the breeds was very slight.

Group E. At 40 days of age (November 24, 1933) 18 Leghorns and 20 Minorcas were parasitized with 50 ± 5 embryonated eggs of *A. lineata.* After three weeks of parasitism (age 61 days) these chickens were sacrificed and the intestinal worms collected. The range of infestation in the Leghorns was one to 16 worms with four chickens free from infestation. The Minorcas' infestation ranged from one to 8 worms and 9 chickens of this breed escaped infestation.

The biometrical analysis of the results shows that the average number of worms in the Leghorns was 4.5, and in the Minorcas 1.95 worms per chick. The difference of 2.55 worms was significant (3.41 times the probable error). In the Leghorns the average length of the worms was 13.09 mm. as compared with a 10.83 mm. average length in the Minorcas. The difference of 2.26 mm. is 3.75 times the probable error of the difference and hence probably significant.

It is to be noted that for the first time in Experiment 4 the White Leghorns were significantly more susceptible to infestation than were the Minorcas. The results of the statistical analysis of the data in Group E are in full accord with those obtained in Experiments 1, 2, and 3.

However, the total results of Experiment 4 are too much at variance with those of the earlier experiments to allow the correlation of one part to be a justifiable or final conclusion.

The evidence of age resistance obtained from the biometrical analysis of the results of Experiment 4 is very contradictory (Table 8). The comparisons of Groups A and B, parasitized at 12 and 19 days and killed at 33 and 40 days respectively; and of Groups B and C, parasitized at 19 and 26 days and killed at 40 and 47 days, respectively, revealed very erratic results for both breeds. The analysis of the Leghorn A and B comparison of numbers of worms gave a negative significance, that is, Group A seemed to be more resistant than Group B which was older when parasitized. There was no significant difference in length of the worms in these Leghorn groups. With the equivalent groups of the Minorcas, the reverse was true. In this breed, the comparison of worm lengths revealed a significant difference in which the longer worms were in the younger group. No constant difference was found in numbers of worms. In the comparison of the B and C Groups parasitized at 19 and 26 days respectively, no constant difference was found in numbers of worms but both breeds showed significant differences in length of parasites, demonstrat-

Table 8. A summary of the numbers and lengths of the Ascaridia lineata from the chickens of Experiment 4 (Fall, 1933) for evidences of age resistance.

Breed	Group	Number of hosts	Average number worms	Dif.	Ratio	Average length worms	Dif.	Ratio
W. Leghorns	A	23	5.5	5.4	4.66	19.63	0.38	0.79
	B	24	10.9			20.01		
	B	24	10.9	0.1	0.61	20.01	2.51	7.23
	C	25	11.0			17.5		
	C	25	11.0	6.5	4.51	17.5	4.41	9.59
	E	18	4.5			13.09		
W. Minorcas	A	21	8.1	2.6	2.31	18.78	1.81	3.95
	B	22	10.7			20.59		
	B	22	10.7	0.9	0.53	20.59	1.79	4.43
	C	24	9.8			18.8		
	C	24	9.8	7.85	5.49	18.8	7.97	14.46
	E	20	1.95			10.83		

ing the presence of age resistance. When Group C was compared with Group E which was parasitized two weeks after Group C, constant differences indicating evidence of age resistance were revealed. It is possible that the mechanism of so-called age resistance is not developed until the chickens are five or six weeks of age. That would account for the very erratic results in the younger groups.

Experiment 5

The White Leghorn and White Minorca chickens used in Experiment 5 were hatched January 14, 1934, and were received the following day. They were secured from the same hatchery as those chickens in the former experiments. Average weekly weights were taken and growth curves of the two breeds compared (Fig. 5). The same discrepancy that was noted in Experiment 4 (Fig. 4), that is, the growth curve of the Minorcas falling well below that of the Leghorns, was evident in Experiment 5. It would seem from these data that the chickens of Experiments 4 and 5 or at least the White Minorcas in these experiments belonged to a different strain than did those chickens of the three former experiments. The varied results from Experiments 4 and 5 when compared with those of Experiments 1, 2, and 3 tend to substantiate the strain theory.

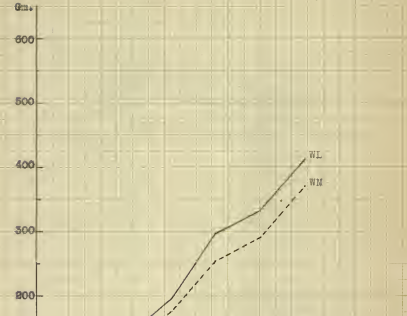

Fig. 5. Showing growth curves of White Leghorns (WL) and White Minorcas (WM). Winter, 1934.

Group A. At 12 days of age, on January 26, 1934, 12 White Leghorns and 11 White Minorcas were parasitized with 50 ± 5 embryonated eggs of A. lineata. The infestation in the Leghorns ranged from one to 11 worms and three chickens of this breed escaped infestation. The range of infestation for the Minorcas was greater, from one to 19 worms. Two of the Minorcas were not infested. The average number of worms in the Leghorns was 3.42; while the Minorcas averaged 6.36 worms per chick, a mean difference of 2.94 that was 2.22 times the probable error. The average length of these Leghorn parasites was 21.95 mm. That of the Minorcas was 24.32 mm., a mean difference of 2.37 that was 2.25 times the probable error. It will be noted that the range of infestation was rather uniform, and that while the differences were not significant, they tend to confirm the results of Experiment 4, Group A, that the Minorcas are possibly less resistant to infestation than are the Leghorns.

Group B. On February 2, 1934, at 19 days of age, 12 White Leghorns and 18 White Minorcas were parasitized with 50 ± 5 embryonated eggs of A. lineata. Three weeks later these chickens were killed and the A. lineata isolated. The range of infestation for the Leghorns was one to 10 worms and three chickens of this breed were not infested.

In the Minoreas the range of infestation was one to 15 worms. Four Minoreas escaped infestation. The average number of worms per Leghorn was 3.92; per Minorca 4.72, an average difference of 0.80 worms that was not significant. The average length of the worms in these Leghorns was 20.98 mm. In the Minoreas the worms averaged 19.56 mm. The mean difference of 1.42 mm. was not significant. The range of infestation was rather uniform for the Leghorns but somewhat less so for the Minoreas. Both breeds, however, appear to be about equally resistant to infestation.

Group D. Twenty-six White Leghorns and 26 White Minoreas were parasitized February 16, 1934, at 33 days of age with 50 ± 5 embryonated eggs of A. lineata. After three weeks of parasitism, the chickens were killed and the A. lineata collected. The range of infestation for the Leghorns was very low, four A. lineata being the largest number found in one chicken. Eleven Leghorns were not infested. The number of worms in the Minoreas ranged from one to 8 and 14 chickens of this breed escaped infestation. The average number of worms in the Leghorns was 1.0; in the Minoreas 1.35, a difference of 0.35 that was not significant. The average length of the worms in the Leghorns was 17.81 mm.; in the Minoreas, 23.74 mm., or a mean difference of 5.93 mm. which was significant (5.59 times the probable error).

The results from Experiment 5 have shown little difference between the resistance to infestation of the Leghorns and the Minoroas (Table 9). Only with regard to the lengths of the worms in Group D of the entire experiment have the differences between the breeds been significant. The analysis of the worm lengths in this case shows that the Minoreas are less resistant to infestation than are the White Leghorns.

The evidences of age resistance resulting from the biometrical analysis of the data from Experiment 5 are contradictory and inconclusive (Table 10). Groups A and B were parasitized at 12 and 19 days of age, and killed after three weeks of parasitism at 33 and 40 days, respectively. Group B was further compared with Group D which was parasitized at 33 days of age and killed three weeks later at 54 days of age. The A-B Leghorn groups revealed no constant difference for either number or lengths of worms. The comparison of the B-D groups of this breed suggests a developing age resistance. There was a constant difference in the average number of worms, while the 3.17 mm. difference in lengths of worms was just short of significance. In the equivalent Minorca group comparisons, the A-B groups showed no significance for numbers of worms, but the lengths of

Table 9. Comparison of Ascaridia lineata found in chickens of Experiment 5, hatched January 14, 1934; Group A parasitised at 12 days, Group B at 19 days, and Group D at 33 days of age; and killed after three weeks of parasitism, respectively.

	Breed	Group	Number of hosts	Mean	Stand. Dev.	Error of mean	Dif.	Probable error of difference	Ratio $\frac{D}{P.E.D.}$
Numbers of Worms	W. Leghorns	A	12	3.42	3.22	.628	2.94	1.33	2.22
	W. Minorcas	A	11	6.36	5.96	1.211			
	W. Leghorns	B	12	3.92	3.04	.593	0.80	0.98	0.819
	W. Minorcas	B	18	4.72	4.88	.776			
	W. Leghorns	D	26	1.0	1.11	.147	0.35	0.32	1.09
	W. Minorcas	D	26	1.35	2.16	.286			
Lengths of Worms (mm.)	W. Leghorns	A	12	21.95	8.15	.859	2.37	1.05	2.25
	W. Minorcas	A	11	24.32	7.54	.608			
	W. Leghorns	B	12	20.98	6.87	.677	1.42	0.83	1.72
	W. Minorcas	B	18	19.56	6.49	.475			
	W. Leghorns	D	26	17.81	6.55	.866	5.93	1.06	5.59
	W. Minorcas	D	26	23.74	5.37	.612			

Table 10. A summary of the numbers and lengths of the *Ascaridia lineata* from the chickens of *Experiment 5* (Winter, 1934) for evidences of age resistance.

Breed	Group	Number of hosts	Average number worms	Dif.	Ratio	Average length worms	Dif.	Ratio
W. Leghorns	A	12	3.42	0.50	0.58	21.95	0.97	0.89
	B	12	3.92			20.98		
	B	12	3.92	2.92	4.78	20.98	3.17	2.88
	D	26	1.0			17.81		
W. Minorcas	A	11	6.36	1.64	1.01	24.32	4.76	6.17
	B	18	4.72			19.56		
	B	18	4.72	3.37	4.07	19.56	4.18	5.40
	D	26	1.35			23.74		

worms revealed a constant difference that suggested evidence of age resistance. There was a significant difference in numbers of worms in the B-D group comparison of this breed also indicative of age resistance, but the analysis of the lengths of the worms gave contradictory results. Here, there was a constant difference in worm lengths, but it was negative, that is, the longer worms were in the older chickens.

As in Experiment 4, it would seem that the mechanism of age resistance is not developed at the earlier ages. Herrick (1926) showed evidence of a gradually increasing age resistance, but he was working with rather small numbers of chickens over a comparatively shorter period. There also seems to be evidence that the White Minorcas have less tendency to develop age resistance than do the Leghorns. In this experiment the Minorcas were more variable than the Leghorns.

DISCUSSION

The general analysis of the results of these experiments indicates that the White Leghorn breed of chickens is less resistant to the intestinal worm, A. lineata than is the breed of White Minorcas. Especially does the early work, that completed prior to the Fall of 1933, bear out this conclusion. The results of the analysis reveal that the differences involved are significant and are consistantly in favor of the Minoreas. A constant difference regarding age resistance is found in both breeds, but especially is this true of the Leghorns. This is in agreement with the work of Herrick (1926).

Experiments begun in the Fall of 1933 and extending to March, 1934, gave very erratic results when the data were analyzed biometrically. In the groups parasitized at 12, 19, 26, and 33 days of age, not once in the analysis of either numbers or lengths of worms were the White Leghorns found to be more susceptible than were the White Minoreas. In fact, in the 12 day group, the Minoreas proved to be significantly less resistant than the Leghorns as judged by numbers of worms. Further indications of the lesser resistance of the Minoreas were found in the analyses of worm lengths for both the 26 and the 33 day groups where constant differences occurred. Other data of the four groups were not significant. Group E, parasitized at 40 days of age was peculiar in this series of experiments because significant differences between the breeds for both numbers and lengths of worms indicated that the Minoreas were more resistant to infestation than were the Leghorns.

The evident contradictions occurring as they did under carefully controlled conditions are best explained by probable innate differences between the breeds or among strains within the breeds. The Leghorns and the Minorcas are both Mediterranean breeds of fowl with the same general body conformation, but the standard weights (Jull, 1926) list White Minorcas one pound heavier than White Leghorns. Both of these breeds are classed as non-broody and are kept for egg-producing purposes rather than for meat production. Observations on the general development revealed neglibible differences between these breeds. Feather-growth, early crowing attempts of young cockerels, and activity of the breeds were so closely correlated that the two breeds of chickens evidently are closely related.

Closer inspection is needed to clarify the conflicting results so that a conclusion on the respective or comparative resistance of these two breeds may be reached. Standard weights of breeds list the White Minorcas as one pound heavier than White Leghorns. Because their egg sizes and their rates of growth are approximately equivalent, it is reasonable to assume that, starting at approximately the same weights, the White Minorcas would build up a slight but increasing lead on the basis of normal growth curves. The first series of experiments (those prior to the Fall of

1933) actually showed such growth curves (Figs. 1, 2 and 3) and it was in these experiments that the Minorcas were more resistant to A. lineata than were the Leghorns. When the growth curves for the experiments conducted in the Fall of 1933 and the Winter of 1934 (Figs. 4 and 5) were graphed it was found that the Minorcas averaged considerably less in weight than the Leghorns throughout the experiments, and it was in these experiments that the Minorcas were less resistant than were the Leghorns. Moreover, when these growth curves from the second series of experiments were superimposed upon those of the first series (Figs. 1, 2, and 3) it was evident that the Minorcas of the second series were markedly lighter in weight. This fact together with the lower resistance of these Minorcas as compared with the earlier ones leads to the inference that different strains of Minorcas were used in the respective series of experiments. On the basis of the results, the presence of strains may account for the major variation between the breeds. On the other hand, it is possible that, with the similarity of these two breeds, the comparison of larger numbers of both breeds would fail to show constant differences in their resistance to A. lineata.

SUMMARY

1. Five experiments involving 470 chickens were conducted to compare the resistance of White Leghorn and White Minorca chickens to the nematode *Ascaridia lineata* (Schneider).

2. The chicks secured at commercial hatcheries were raised in confinement. All groups under comparison were of the same ages and were given the same number (50 ± 5) of nematode eggs. The criteria for judging the resistance were the numbers (viability) and the lengths (growth) of the *A. lineata* from each group of chickens.

3. The results of Experiments 1, 2, and 3 (Fall, 1932; Winter, 1933; and Summer, 1933, respectively) indicated that the White Leghorns were less resistant to the nematodes than were the White Minorcas. In Experiments 4 and 5 (Fall, 1933, and Winter, 1934) the failure of the results to fully substantiate the earlier ones is attributed in part to the smaller White Minorcas used. The results of the five experiments indicate that White Minorca chickens are more resistant to *A. lineata* than are White Leghorns when birds of normal weight are used; when White Minorcas of subnormal weight are compared with Leghorns of normal weight the latter appear to be more resistant to the parasites. Apparently two

strains of White Minorcas were used.

4. The development of age resistance was more rapid in the White Leghorns than in the White Minorcas; it was less marked in chickens parasitized at 33 days of age or younger than among chickens parasitized at older ages. The variability in both numbers and lengths of nematodes increases in chickens parasitized at five or more weeks of age.

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